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ARTICLE

Serum Neuron-Specific Enolase and High-Sensitivity C-Reactive Protein Expression Levels and Their Clinical Significance in Patients With Alzheimer's Disease

Zhangning Zhou[1], Feimin Zhao[1,*]

[1]Department of Geriatric Medicine, Huzhou Central Hospital, Affiliated Central Hospital Huzhou University, Huzhou, Zhejiang, China

*Correspondence: feimin_zhao@126.com (Feimin Zhao)

Abstract

Aims/Background Alzheimer's disease (AD) is a degenerative disease of the central nervous system. Identifying effective and highly specific serum biomarkers is crucial for the early diagnosis and therapeutic monitoring of AD. This study aimed to explore the serum levels of neuron-specific enolase (NSE) and high-sensitivity C-reactive protein (hs-CRP) and their clinical significance in AD patients.

Methods This retrospective study recruited 112 AD patients hospitalized between June 2021 and June 2023 as an AD group. For comparison, 80 healthy individuals who underwent physical examination during the same period were selected as the control group. The levels of NSE and hs-CRP were assessed using enzyme-linked immunosorbent assay (ELISA). The levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were also determined using ELISA. Furthermore, the severity of cognitive impairment was evaluated using the Mini-mental State Examination (MMSE) score, the Global Deterioration Scale (GDS), and the Clinical Dementia Rating Scale (CDR). Pearson correlation analysis was used to analyze the correlation between serum NSE and hs-CRP levels and disease-related indicators in the AD group, and the receiver operating characteristic (ROC) was used to analyze their diagnostic efficacy.

Results The AD group exhibited significantly higher GDS and CDR scores, as well as serum NSE and hs-CRP levels, and significantly lower MMSE scores compared to the control group ($p < 0.001$). GDS and CDR scores, and serum NSE and hs-CRP levels were significantly higher in the moderate-to-severe group than in the mild group, and significantly lower MMSE scores ($p < 0.001$). Pearson correlation analysis revealed that serum NSE and hs-CRP levels were negatively correlated with MMSE scores in AD patients ($p < 0.05$) and were positively correlated with GDS and CDR scores ($p < 0.05$). ROC curve analysis showed that the serum NSE (area under the curve [AUC]: 0.856, 95% CI 0.787–0.925, $p < 0.001$) and hs-CRP (AUC: 0.728, 95% CI 0.631–0.825, $p < 0.001$) levels individually had significant diagnostic efficacy for AD; however, the combined assessment of their levels (AUC: 0.879, 95% CI 0.815–0.943, $p < 0.001$) demonstrated higher diagnostic efficacy than hs-CRP alone ($p < 0.001$).

Conclusion Serum NSE and hs-CRP levels are closely associated with the cognitive function in AD patients, and their combined evaluation exhibits a higher diagnostic value.

Key words: Alzheimer's disease; neuron-specific enolase; high-sensitivity C-reactive protein

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Introduction

Alzheimer's disease (AD) is a prevalent type of dementia, comprising 60 to 80 percent of all cases (Rostagno, 2022). With the aging of global population, the

incidence of AD continues to increase each year. A survey reported an incidence rate of 11.3% in individuals aged ≥65 years, with a mortality rate of about 37.0%, making it the fifth major cause of death (Alzheimer's Association, 2021). The common clinical manifestations of AD include cognitive dysfunction, persistent memory loss, and behavior abnormalities (Qin et al, 2020). Current understanding of AD pathogenesis involves mitochondrial dysfunction, the amyloid hypothesis, oxidative stress-induced damage, and neuroinflammation (Sharma et al, 2024; Chen et al, 2020). Moreover, age-related neurotransmitter changes are also crucial (Li et al, 2023). Furthermore, its primary pathological characteristics include the formation of senile plaques, which are composed of β-amyloid and neurofibrillary tangles resulting from abnormal phosphorylation of tau protein (Roda et al, 2022).

Current AD treatment modalities mainly aim to improve cognitive function and alleviate symptoms; however, they do not prevent disease progression (Breijyeh and Karaman, 2020). Diagnostic approaches like cerebrospinal fluid (CSF) analysis for β-amyloid (Aβ) and tau protein, along with neuroimaging techniques such as magnetic resonance imaging (MRI), tau protein positron emission tomography (tau-PET) and Aβ PET, have facilitated early identification and therapeutic monitoring of AD. However, these methods are limited by their invasive nature, high costs, and insufficient accessibility to specialized equipment (Kim et al, 2021). In recent years, research on blood biomarkers has opened promising avenues for non-invasive diagnosis of AD (Molinuevo et al, 2018). For example, increased levels of neurofilament light chain (NfL) and total tau protein have demonstrated strong diagnostic potential in distinguishing AD from cognitively healthy individuals (Hampel et al, 2018; Khalil et al, 2018). However, their limited specificity decreases their efficacy in differentiating AD from other neurodegenerative diseases (Abed et al, 2023). Therefore, identifying blood biomarkers with high sensitivity and specificity remains challenging in AD research.

Furthermore, neuron-specific enolase (NSE) is a dimeric isoenzyme of soluble cytoplasmic protein enolase, playing a crucial role in maintaining neuronal activity (Rajguru et al, 2020). High-sensitivity C-reactive protein (hs-CRP) is a highly sensitive biomarker of inflammatory response, which may affect cognitive function by disrupting vascular endothelial function, inhibiting angiogenesis and promoting inflammatory response (Yang et al, 2020). As a typical indicators reflecting neuroinflammation, hs-CRP can stimulate phagocytosis and activate the complement system (Banait et al, 2022). Research has proved that hs-CRP levels can affect the clearance of Aβ plaques by microglia, contribute to neuronal degeneration and death, and are strongly linked to the severity of AD (Naomi et al, 2021). Additionally, elevated NSE expression has been found in the cerebrospinal fluid of AD patients, suggesting its potential as a novel marker for early diagnosis (d'Abramo et al, 2020). When brain tissue is damaged, changes in the permeability of cell membranes facilitate NSE to pass through the blood-brain barrier into the bloodstream, resulting in increased peripheral blood levels (Lasek-Bal et al, 2019). A recent study also suggests that elevated serum hs-CRP levels are associated with an increased risk of dementia, especially AD, in community-dwelling elderly populations in Japan (Tachibana et al, 2024). Therefore, NSE and hs-CRP in peripheral

blood can be promising biomarkers for early detection and progression monitoring of AD (Chen et al, 2023; Zhang et al, 2022).

Therefore, this study aimed to investigate the clinical significance of serum NSE and hs-CRP expression levels in AD patients. The findings would provide valuable insights for the early diagnosis and evaluation of treatment outcomes in AD patients.

Methods

Recruitment of Research Participants

This retrospective study enrolled 112 AD patients hospitalized between June 2021 and June 2023 in Huzhou Central Hospital. The control group (n = 80) was composed of healthy individuals who underwent physical examination during the same period, excluding those with suspected dementia. This research was approved by the Medical Ethics Committee of Huzhou Central Hospital (Approval number: 2023-12-27), and informed consent was obtained from the patients or their families. The study design adhered to the principles of the Declaration of Helsinki.

Inclusion criteria for patient selection were as follows: (1) Patients qualifying the diagnostic criteria outlined in the "*China Guidelines for the Diagnosis and Treatment of Dementia and Cognitive Impairment* (2): *Guidelines for the Diagnosis and Treatment of Alzheimer's Disease* (2018)" (Chinese Dementia and Cognitive Disorders Writing Group and Cognitive Disorders Disease Professional Committee of Neurology Branch of Chinese Medical Doctor Association, 2018). (2) Patients diagnosed as non-vascular dementia confirmed through head magnetic resonance imaging (MRI). (3) Those aged >18 years. (4) Individuals with stable mental states and capable of independently completing the assessment scales. (5) Those with complete clinical records.

Patient exclusion criteria included: (1) Those with other types of dementia caused by drugs, infection, or trauma. (2) Individuals diagnosed with other mental illnesses. (3) Patients with significant abnormalities of vital organs such as the heart, liver, kidneys, lungs, or other severe medical conditions. (4) Those with congenital abnormalities in the development of the nervous system. (5) Patients diagnosed with severe infections, systemic immune disorders, or malignant neoplasms. (6) Those with impairments in vision, hearing, and other sensory functions, rendering them unable to complete the assessments.

In the control group, individuals with AD or other degenerative diseases of the central nervous system (CNS) were excluded. However, all other criteria for the control group were consistent with those used for the AD group.

Baseline Characteristic

Baseline demographic and clinical characteristics were collected from all subjects through interviews during admission or by reviewing their medical records. These characteristics included age, sex, smoking history (smoking at least one cigarette a day for >6 months or meeting this criterion in the past, with cessation occurring within the last six months) (Yan et al, 2024), and alcohol consumption

history (drinking alcohol at least once a week for more than 6 months, or meeting this criterion in the past with abstinence of less than 6 months) (Yan et al, 2024). Other baseline variables included education level, whether the patient lived alone, and the presence of cardiovascular disease (defined as a history of congestive heart failure, coronary heart disease, angina, or myocardial infarction) (Zhu et al, 2020). A history of cerebrovascular disease was also recorded, such as cerebral hemorrhage, transient ischemic attack, subarachnoid hemorrhage, and/or ischemic stroke (Matsufuji et al, 2021; Miyabe et al, 2019). Family history of AD was documented if a first-degree relative (direct descendant or sibling) had been diagnosed with AD (Donix et al, 2012). Furthermore, history of hypertension defined as a self-reported history of high blood pressure and the use of antihypertensive medications (Feng et al, 2022), and history of diabetes defined as a self-reported history of diabetes mellitus or use of antidiabetic medications (Chang et al, 2019) were collected.

Cognitive Impairment Score

The severity of cognitive impairment was determined using the Mini-mental State Examination (MMSE) score (Folstein et al, 1983), the Global Deterioration Scale (GDS) (Reisberg et al, 1982), and the Clinical Dementia Rating Scale (CDR) (Morris, 1993).

• The GDS classified the severity of dementia on a scale ranging from 1 to 7, where scores of 1 indicating no cognitive impairment, 2 indicating subjective memory complaints, 3 corresponds to mild cognitive impairment, and a score from 4 to 7 represents progressive stages of dementia from mild to severe.

• In the MMSE scale, the total score ranges from 0 to 30, with the interpretation as follows: scores from 0 to 10 indicate severe dementia, 11 to 20 indicate moderate dementia, 21 to 26 indicate mild dementia, and 27 to 30 indicate no dementia symptoms.

• The CDR assessment involves scores of 0, 0.5, 1, 2, and 3 points, which represent normal cognition, suspicious, light, medium, and severe dementia, respectively.

Based on MMSE, GDS, and CDR scores, patients in the AD group were further divided into two subgroups: a mild group (n = 64; MMSE score: 21–26, CDR score: 0.5 or 1, GDS score: 3 or 4), and a moderate-to-severe group (n = 48; MMSE score: 0–20, CDR score: 2 or 3, GDS score: 5–7). Scoring was conducted by professionally qualified neurologists who have undergone specialized training in administering and interpreting these cognitive assessment tools. The neurologists were blinded to the subjects' baseline data.

Detection of NSE and hs-CRP Levels in Serum

Blood samples were collected from all participants between 7 AM and 9 AM, after a fasting period of at least 12 hours. Fasting was defined as not consuming food or drinks except water for at least 12 hours before blood collection. Adherence to fasting was confirmed through self-report and interviewer verification. A 5 mL venous blood was obtained from each participant in a vacuum blood collection tube without anticoagulant. Subsequently, the blood samples were centrifuged (Allegra

X-30R, BECKMAN, Brea, CA, USA) at 3000 rpm with a centrifuge radius of 10 cm for 15 minutes. Following centrifugation, the upper layer of serum was carefully extracted and immediately stored at –70 °C in a freezer (Thermo Fisher Scientific, Waltham, MA, USA) to ensure that the total time between blood collection and biomarker measurement did not exceed 24 hours.

Serum NSE levels were assessed using the NSE enzyme-linked immunosorbent assay (ELISA) kit (QT13290, Shanghai Qitai Biotechnology Co., Ltd., Shanghai, China) at room temperature equilibrium. Similarly, the level of hs-CRP in serum was assessed using corresponding ELISA kit (ml092638, Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). All procedures were performed following the manufacturer's instructions.

Evaluation of Serum TG, TC, HDL-C, and LDL-C Levels

Serum samples were collected for all participants, as described in the section "Detection of NSE and hs-CRP Levels in Serum". Corresponding ELISA kits were used to assess the serum levels of total cholesterol (TC) (BC1985, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), triglyceride (TG) (BC0620, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), high-density lipoprotein cholesterol (HDL-C) (BC5320, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), and low-density lipoprotein cholesterol (LDL-C) (BC5330, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), following the manufacturer's instructions. Briefly, 25 μL of sample and standard calibrator were added to the designated wells and plates were sealed with cover film. After incubation for 30 minutes, 250 μL of enzyme-labeled antibody working solution and substrate were added to each well, mixed well by pipetting, and incubated at 37 °C for 60 minutes. The reaction was terminated after the given time. A standard curve was developed by plotting the optical density (OD) values of the standard substrate solution against their known concentrations. Using the linear regression equation, the levels of the TC, TG, HDL-C, and LDL-C in the samples were determined based on their OD values.

Statistical Analysis

Statistical analysis was performed using SPSS 23.0 software (IBM, Armonk, NY, USA). Kolmogorov-Smirnov was used to test the normality in measurement data. Measurement data following normal distribution were presented as mean ± standard deviation ($\bar{x} \pm s$), with comparisons between groups performed using the independent sample *t*-test. However, count data were represented as percentages, n (%), and analyzed using the χ^2 test. In the χ^2 test, when expected frequencies (T) were $\geq$5 and total samples (n) were $\geq$40, then the Pearson χ^2 test was applied. If $1 \leq T < 5$ with $n \geq 40$, the χ^2 test with Yates's correction was used. Pearson correlation analysis was used to examine the correlation between serum NSE and hs-CRP levels and disease-related indicators in AD patients. Furthermore, receiver operating characteristic (ROC) curves were used to analyze the diagnostic value of serum NSE and hs-CRP levels, both individually and in combination. A *p*-value of <0.05 were considered statistically significant.

Table 1. Comparison of clinical data and cognitive impairment scores between the two groups [$\bar{x} \pm s$, n (%)].

Variables	AD group (n = 112)	Control group (n = 80)	χ^2/t-value	p-value
Gender			0.091	0.762
Male	43 (38.39)	29 (36.25)		
Female	69 (61.61)	51 (63.75)		
Age (years)	69.71 ± 7.15	71.03 ± 6.68	1.296	0.197
Smoking history, yes	27 (24.11)	20 (25.00)	0.020	0.887
Drinking history, yes	31 (27.68)	21 (26.25)	0.048	0.826
Education level (years)	8.59 ± 2.67	8.41 ± 2.12	0.501	0.617
Live alone	47 (41.96)	30 (37.50)	0.387	0.534
TC (mmol/L)	4.61 ± 2.09	4.17 ± 2.31	1.376	0.170
TG (mmol/L)	1.84 ± 0.90	1.68 ± 0.79	1.277	0.203
HDL (mmol/L)	1.37 ± 0.55	1.34 ± 0.38	0.421	0.674
LDL (mmol/L)	2.73 ± 1.35	2.66 ± 1.17	0.374	0.709
MMSE (points)	20.65 ± 4.40	28.75 ± 2.04	15.323	<0.001
GDS (points)	4.47 ± 1.07	1.23 ± 0.42	25.691	<0.001
CDR (points)	1.48 ± 0.39	0	33.917	<0.001
NSE (μg/L)	16.34 ± 3.87	9.88 ± 1.72	13.969	<0.001
hs-CRP (mg/L)	6.57 ± 2.25	0.83 ± 0.54	22.347	<0.001
Past medical history				
Cardiovascular disease	20 (17.86)	11 (13.75)	0.581	0.446
Cerebrovascular disease	14 (12.50)	10 (12.50)	<0.001	1.000
Family history of AD	4 (3.57)	2 (2.50)	<0.001	1.000
Hypertension	25 (22.32)	15 (18.75)	0.361	0.548
Diabetes	14 (12.50)	9 (11.25)	0.069	0.793

Note: AD, Alzheimer's disease; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MMSE, Mini-mental State Examination; GDS, Global Deterioration Scale; CDR, the Clinical Dementia Rating Scale; NSE, neuron-specific enolase; hs-CRP, high-sensitivity C-reactive protein.

Results

Comparison of Clinical Data and Cognitive Impairment Scores Between the Control and AD Groups

The two groups showed no significant differences in the baseline data, including gender distribution, age, smoking history, drinking history, education level, solitary condition, serum TG, TC, HDL-C, and LDL-C levels and past medical history ($p > 0.05$), indicating comparability between them. However, GDS and CDR scores, as well as serum NSE and hs-CRP levels, were significantly higher in AD group than in control group ($p < 0.001$). In contrast, the AD group exhibited a considerably lower MMSE score than the control group ($p < 0.001$, Table 1).

Table 2. Comparison of clinical data and cognitive impairment scores between mild and moderate-to-severe patients [$\bar{x} \pm s$, n (%)].

Items	Mild group (n = 64)	Moderate-to-severe group (n = 48)	χ^2/t-value	p-value
Gender			0.028	0.866
Male	25 (39.06)	18 (37.50)		
Female	39 (60.94)	30 (62.50)		
Age (years)	69.47 ± 5.73	70.18 ± 5.54	0.658	0.512
Smoking history, yes	16 (25.00)	11 (22.92)	0.065	0.799
Drinking history, yes	17 (26.56)	14 (29.17)	0.093	0.760
Education level (years)	8.23 ± 2.15	8.84 ± 2.32	1.436	0.154
Live alone	25 (39.06)	22 (45.83)	0.516	0.472
TC (mmol/L)	4.44 ± 1.61	4.83 ± 1.15	1.427	0.157
TG (mmol/L)	1.78 ± 0.52	1.92 ± 0.46	1.480	0.142
HDL (mmol/L)	1.35 ± 0.31	1.40 ± 0.37	0.777	0.439
LDL (mmol/L)	2.82 ± 0.76	2.61 ± 0.93	1.314	0.192
MMSE (points)	23.17 ± 4.58	18.04 ± 3.19	6.642	<0.001
GDS (points)	3.48 ± 0.50	5.77 ± 0.81	18.429	<0.001
CDR (points)	0.78 ± 0.25	2.42 ± 0.50	22.744	<0.001
NSE (μg/L)	14.19 ± 3.74	18.48 ± 4.02	5.817	<0.001
hs-CRP (mg/L)	5.86 ± 1.97	7.97 ± 2.51	4.985	<0.001
Past medical history				
Cardiovascular disease	12 (18.75)	8 (16.67)	0.081	0.776
Cerebrovascular disease	8 (12.50)	6 (12.50)	<0.001	1.000
Family history of AD	2 (3.13)	2 (4.17)	<0.001	1.000
Hypertension	14 (21.88)	11 (22.92)	0.017	0.896
Diabetes	7 (10.94)	7 (14.58)	0.333	0.564

Comparison of Clinical Data and Cognitive Impairment Scores Between Mild and Moderate-to-Severe Groups

GDS and CDR scores, as well as serum NSE and hs-CRP levels, were significantly higher in the moderate-to-severe group than in the mild group ($p < 0.001$). However, the moderate-to-severe group had significantly lower MMSE ($p < 0.001$). As shown in Table 2, the two groups demonstrated no significant differences in other indexes ($p > 0.05$).

Correlation Between Serum NSE and hs-CRP Levels and Cognitive Impairment in AD Patients

Pearson correlation analysis revealed that serum NSE and hs-CRP levels were negatively correlated with MMSE scores in AD patients ($p < 0.05$). However, serum NSE and hs-CRP levels demonstrated positive correlations with GDS and CDR scores in AD ($p < 0.05$, Table 3).

Diagnostic Efficacy of Serum NSE and hs-CRP Levels in AD

ROC curve results showed that the area under the curve (AUC) for serum NSE, hs-CRP, and their combined assessment were 0.856 (95% CI: 0.787–0.925, $p <$

Table 3. A correlation between serum NSE and hs-CRP levels and cognitive impairment in AD patients (n = 112).

Items	MMSE		GDS		CDR	
	r	*p*-value	r	*p*-value	r	*p*-value
NSE	–0.357	<0.001	0.371	<0.001	0.360	<0.001
hs-CRP	–0.213	0.024	0.223	0.018	0.288	0.002

0.001), 0.728 (95% CI: 0.631–0.825, $p < 0.001$) and 0.879 (95% CI: 0.815–0.943, $p < 0.001$), respectively (Fig. 1, Table 4). Although the combined detection of NSE and hs-CRP did not significantly improve predictive efficacy compared to NSE alone ($p = 0.280$), it showed substantially higher predictive performance than hs-CRP alone ($p < 0.001$).

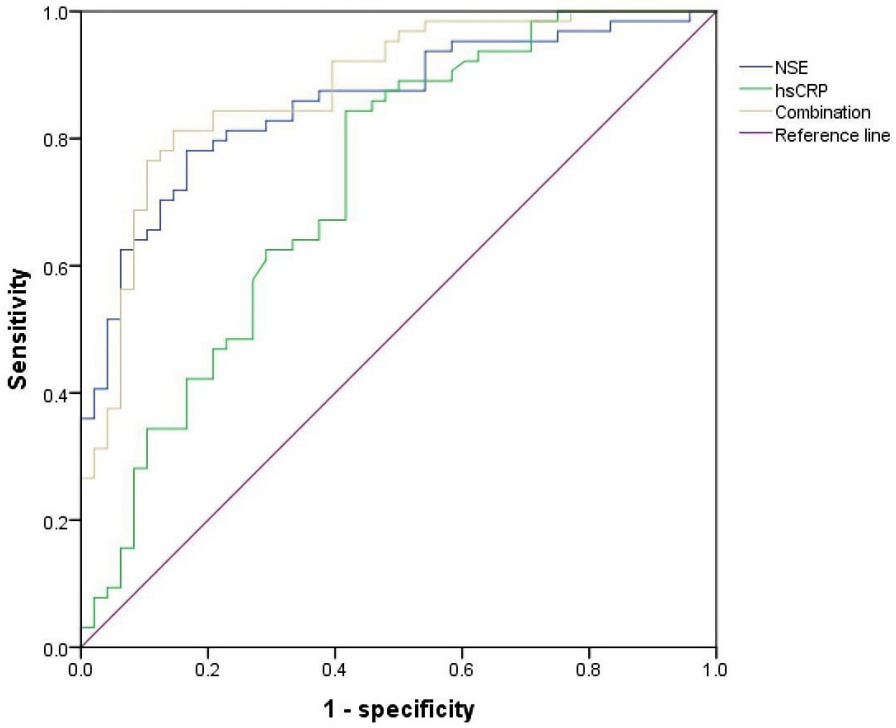

Fig. 1. ROC curve analysis of serum NSE and hs-CRP levels in diagnosis of AD. ROC, receiver operating characteristic.

Discussion

The pathological mechanism of AD remain complex, and effective approaches for its prevention and treatment are still limited (Zhang et al, 2024). Exploring key molecular markers involved in the occurrence and development of AD is therefore crucial for enhancing early clinical diagnosis and management. In this study, the serum NSE and hs-CRP expression levels in AD patients demonstrated a significant correlation with cognitive impairment, as evidenced by MMSE, GDS, and CDR scores. Moreover, the diagnostic efficacy assessment using ROC curve analysis

Table 4. Diagnostic performance of serum NSE and hs-CRP levels alone and in their combined assessment in AD (n = 112).

Items	Cut-off value	AUC	*p*-value	95% CI	Sensitivity	Specificity	Youden index
NSE	16.26	0.856	<0.001	0.787–0.925	0.781	0.833	0.614
hs-CRP	7.49	0.728	<0.001	0.631–0.825	0.844	0.583	0.427
Combination	22.79	0.879	<0.001	0.815–0.943	0.813	0.854	0.667

Note: AUC, area under the curve.

revealed that NSE and hs-CRP individually had strong predictive values, with their combined detection offering greater diagnostic performance for AD.

NSE is an enolase involved in the glycolysis pathway, predominantly observed in neuronal brain cells. When neurons are damaged, serum NSE levels increase significantly (Vlasakova et al, 2023). Previous research has shown a clear correlation between NSE levels and dysfunctions in learning, memory, and executive abilities in patients with postoperative cognitive impairment (Park et al, 2020). Both biological and some non-biological factors can stimulate neuroinflammation, releasing neuroinflammatory mediators that affect the level of NSE and aggravate neuronal damage (Pan et al, 2022). In this study, serum NSE levels were substantially elevated in AD patients compared to the non-AD population, suggesting ongoing neuronal damage in individuals with AD.

Furthermore, hs-CRP is a non-specific marker synthesized by the liver in response to systemic inflammation. hs-CRP levels increase rapidly within a few hours after inflammation or injury and decrease as the disease improves, rendering it a widely used clinical indicator (Ghanbari et al, 2023). In AD patients, hs-CRP has been proven to promote the deposition of extracellular amyloid plaques and accelerate disease progression by modulating inflammatory mediator levels (Kesika et al, 2021). Additionally, hs-CRP can activate monocytes and macrophages, inducing them to release pro-inflammatory factors and matrix metalloproteinases, leading to neuroinflammatory damage (Sun et al, 2023). Consistent with the findings of a meta-analysis showing persistently elevated peripheral CRP levels in AD patients, we observed substantially higher serum hs-CRP levels in the AD group compared to the control group, supporting the presence of chronic neuroinflammation in AD (Su et al, 2019).

In our study, MMSE scores were significantly lower in the AD group than in the control group, while GDS and CDR scores were higher, indicating central nervous system dysfunction, cognitive impairment, and significant neuropsychiatric symptoms. Previous studies have reported that elevated hs-CRP levels can also cause endothelial cell damage and promote atherosclerosis, which may further aggravate cognitive impairment (Banait et al, 2022; Wang et al, 2022). Similarly, an association between higher serum NSE levels and cognitive performance has been previously established, with higher levels worsening cognitive impairment (Chaves et al, 2010). The results of our study demonstrated that serum NSE and hs-CRP levels in AD patients had a positive correlation with GDS and CDR scores and showed negative correlation with MMSE scores. These findings suggest that ele-

vated serum NSE levels may reflect the extent of neuronal damage, while increased hs-CRP may be indicative of underlying neuroinflammatory responses. Therefore, the combined rise of these two biomarkers could serve as a potential indicator of cognitive decline in AD patients. Furthermore, ROC curve analysis showed that the AUCs for NSE and hs-CRP individually and their combined assessment were 0.856, 0.728, and 0.879, respectively. The combined prediction approach revealed significantly higher diagnostic performance than hs-CRP alone, indicating high sensitivity (81.3%) and specificity (85.4%). Notably, the diagnostic accuracy of the combined approach (AUC: 0.879) in our study is consistent with that of Lumipulse CSF A$\beta_{42/40}$ (AUC: 0.879), which is currently considered the biomarker with the highest diagnostic accuracy and consistency with amyloid PET (Dakterzada et al, 2021; Lewczuk et al, 2017). These observations further validate the effectiveness and clinical utility of the combined serum-based biomarker method adopted in our study for the early diagnosis of AD.

As medical research advances, there is increasing evidence that the pathophysiology of AD is highly complex and dynamic. AD manifests by the early accumulation of Aβ in the brain, followed by the deposition of tau protein and progressive neurodegenerative changes (Jack et al, 2013). These pathological changes can be detected through CSF analysis, PET, and MRI. Despite their promising insights, the clinical significance of these approaches is often limited due to their invasive nature, high cost, or time-consuming procedure, making them less accessible for routine examination or follow-up assessments in the same individuals (Molinuevo et al, 2018). Compared to these traditional detection methods mentioned, plasma biomarker analysis offers several advantages for AD diagnosis, including being rapid, non-invasive, cost-effective, and having good patient compliance (Hampel et al, 2018). Based on the results of our study, the combined evaluation of serum NSE and hs-CRP provides a potential alternative. With its cost-effectiveness and ease of implementation, this combined approach has the potential to serve as a first-line screening tool for Alzheimer's disease, especially in primary care settings or areas with limited advanced neuroimaging techniques, providing strong support for early AD diagnosis and management (Pereira et al, 2021).

Despite some promising findings, this study has certain limitations. Firstly, the participants were only assessed once, which increases the probability of interference due to laboratory assessment errors, potentially impacting the reliability of the results. Secondly, the retrospective study design limits the ability to eliminate potential biases or the influence of unmeasured confounding factors, which may restrict the reliability of the research findings. Moreover, due to the relatively small sample size and difficulty in data acquisition, a detailed analysis of the impact of comorbidities on serum NSE and hs-CRP levels was not conducted. Additionally, the potential effects of medications could not be completely excluded. Consequently, the observed differences in NSE and hs-CRP levels and cognitive function among various patient stages, such as mild, moderate, and severe, should be interpreted with caution, accounting for the possible impact of unrecorded medication use. To address these limitations, future studies should perform large-scale prospective cohort studies with extensive sample sizes to enhance the universality and reliability

of results. Increasing the number of participant assessments would help reduce single-timepoint assessment errors. Furthermore, more comprehensive and accurate documentation and control of confounding factors, such as comorbidities and drug use, are crucial. Using multi-factor analysis would allow for a thorough evaluation of the role of NSE and hs-CRP in the severity and cognitive function of AD patients.

Conclusion

In summary, serum NSE and hs-CRP levels are strongly linked to cognitive function in AD patients, and their combined detection shows higher diagnostic value. The combined assessment holds considerable diagnostic potential for AD and could offer insights into its prevention and management. Nonetheless, the limited sample size restricts this study, and dynamic fluctuations in serum levels during AD progression or treatment were not assessed. Future studies should prioritize expanding sample sizes and longitudinal designs to enable more in-depth analysis and validation of these biomarkers.

Key Points

- NSE and hs-CRP levels are significantly elevated in AD patients compared to the control group, indicating the presence of neuronal damage and enhanced systemic inflammatory responses in these patients.
- Patients with moderate-to-severe AD exhibit higher NSE and hs-CRP levels compared to those with mild AD, associating disease progression with the cumulative effects of neurodegenerative damage and chronic neuroinflammation.
- Cognitive function, as measured through the MMSE score, is significantly lower in AD patients than in non-AD individuals, while the severity of dementia, as assessed by the CDR and GDS scores, is considerably higher, suggesting that cognitive decline and functional impairment are key clinical features of AD.
- Patients with moderate-to-severe AD show significantly higher CDR and GDS scores and lower MMSE scores than those with mild AD, indicating more severe cognitive impairment and functional disability.
- There is a negative correlation between serum NSE and hs-CRP levels and MMSE scores, and a positive correlation with GDS and CDR scores, indicating that a higher degree of neuronal damage and inflammation are associated with poorer cognitive function and greater severity of dementia.
- The combined detection of serum NSE and hs-CRP levels offers higher diagnostic potential for AD.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

ZZ and FZ designed the research study and wrote the first draft. ZZ and FZ performed the research. ZZ and FZ analyzed the data. Both authors contributed to important editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This research was approved by the Medical Ethics Committee of Huzhou Central Hospital (Approval number: 2023-12-27), and informed consent was obtained from the patients or their families. The study design adhered to the principles of the Declaration of Helsinki.

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Conflict of Interest

The authors declare no conflict of interest.

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